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In Vitro assessment of the survivability of *Lactobacillus casei* DN-114 001 and *Lactobacillus casei* Shirota, within commercialised food matrices, in the upper gastrointestinal tract

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Abstract

The aim of this study was to determine the survivability of probiotics; *Lactobacillus casei* Shirota and *Lactobacillus casei* DN-114 001, within their respective commercialised food matrices Yakult® and Actimel®, during *in vitro* simulated gastrointestinal transit. Original and Light/Fat Free varieties of each brand were assessed to determine whether nutritional composition affected bacterial survival rate. Strains were exposed to 3 hours of simulated gastric transit at pH 2, using Hydrochloric Acid (32%) and Pepsin (3mg ml^{-1}), followed by 2 hours of simulated duodenal transit, at pH 6.5, using Bile Salts (0.3% w/v) and Sodium Hydroxide (4M). Samples were serially diluted with PBS, spread plated onto MRS Agar and incubated at 37^{o}C for 72 hours in a Carbon dioxide incubator (5%). Strains within all products retained viability after 5 hours of simulated gastrointestinal transit. Reductions in *L. casei* Shirota, within Yakult® products (0.993 ± 0.220; Mean ± Standard Deviation), were significantly greater ($P<0.05$), after 5 hours of simulated gastrointestinal transit, than reductions in *L. casei* DN-114 001 within Actimel® products (0.330 ± 0.129). No significant correlation was observed between the variety of brand (Original or Light/Fat Free) and bacterial survival rate after 5 hours of simulated gastrointestinal transit. Results suggest *L. casei* DN-114 001 is more capable at adapting to and surviving the inhospitable conditions of the gastrointestinal tract. However, food matrix composition may have some effect on bacterial survival as reductions of *L. casei* DN-114 001, within Actimel® Fat Free (0.428 ± 0.059), after 5 hours of simulated gastrointestinal transit were proven significant ($P<0.05$), but were insignificant for *L. casei* DN-114 001 within Actimel® Original, when compared to controls. This study demonstrates that *L. casei* Shirota and *L. casei* DN-114 001, in Original and Light/Fat Free versions of Yakult® and Actimel®, have the potential to survive the upper gastrointestinal tract.

Highlights

- All strains retained viability after 5 hours of *in vitro* gastrointestinal transit
- *L. casei* DN-114 001 in Actimel® Original showed resistance to gastrointestinal transit
- *L. casei* Shirota in Yakult® products was significantly affected by gastrointestinal transit
- Composition of food matrix is potentially correlated with bacterial survivability

Keywords: Probiotic, *Lactobacillus casei* Shirota, *Lactobacillus casei* DN-114 001, gastrointestinal tract

Introduction

Probiotics have been defined as "living microorganisms, which upon ingestion in certain numbers exert health benefits beyond inherent basic nutrition" (Guarner & Schaafsma, 1998). The potential therapeutic use of bacteria was first proposed by Metschnikoff (1907) and it is now believed that bacterial probiotics exert health benefits by positively influencing the balance of microflora in the human gastrointestinal tract. Lactic acid bacteria, primarily of the genus *Lactobacillus*, have been assessed extensively for their probiotic potential. Certain *Lactobacillus* species have been shown to antagonise the growth of gastroenteric pathogens, such as *Salmonella typhimurium*, *Helicobacter pylori* and *Pseudomonas aeruginosa* (Bernet-Camard *et al*, 1997; Sgouras *et al*, 2004). Furthermore, species of *Lactobacillus* reportedly enhance the efficacy of human gut-associated lymphoid tissue by augmenting non-specific and specific host immune responses (Gill, 1998). Other proposed health benefits include the prevention of diarrhoeal diseases, anti-tumour action, cholesterol reduction, alleviation of allergic reactions and improvement of lactose utilisation (Kailasapathy & Chin, 2000). Due to the vast range of potential therapeutic benefits, several *Lactobacillus* cultures have become commercialised as pharmaceuticals, nutritional supplements and functional foods; all of which have attached probiotic claims. For example, fermented milk products, such as Yakult® and Actimel®, contain *L. casei* Shirota and *L. casei* DN-114 001 (also known as *L. casei immunitas*) respectively, both of which claim to support the indigenous microflora present in the human gut. However, several remain sceptical of the probiotic functionality of commercialised products, primarily due to the physical and chemical barriers the bacterium has to overcome in order to exert their proposed benefits. The bacterium has to remain viable and active during gastrointestinal transit, where the acidic conditions of the stomach and secretions of hydrolytic enzymes and bile in the small intestine decrease its chance of survival. The bacterium must then defy the digestive flux of the human intestines and out-compete indigenous microflora to adhere to intestinal epithelia. Several studies have assessed the ability of *Lactobacillus* species to resist *in vitro* simulated gastrointestinal tract conditions and adhere to intestinal epithelia. Maragkoudakis *et al* (2006) examined 29 *Lactobacillus* strains of dairy origin for their ability to survive *in vitro* gastric conditions and to adhere to CaCO-2 cells. Interestingly, it was demonstrated that *L. casei* Shirota and *L. casei* DN-114 001 retained no viability after 3 hours of exposure to pH 2 plus pepsin, or after 1 hour of exposure to pH 1. Contrarily, using an *in vitro* dynamic gastric model of digestion, Curto *et al* (2011) showed that *L. casei* Shirota and *L. casei* DN-114 001 both exhibited high tolerance to gastric and duodenal digestion and furthermore, both strains exhibited higher survival rates when milk matrices were used as opposed to water. The major limitation of most studies, assessing gastrointestinal transit tolerance of commercialised probiotic bacteria, is that they are not assessed within the food matrix they are sold in and furthermore, the majority of studies assess resistance to gastric and duodenal transit separately; simulating *in vivo* digestion inadequately.

The aim of this study was to determine the survivability of *L. casei* Shirota and *L. casei* DN-114 001, in their respective Yakult® and Actimel® commercial food matrices, during simulated *in vitro* gastric and subsequent duodenal digestion. Additionally, Original varieties and Light/Fat Free varieties of each probiotic brand were tested to determine if nutritional content affected the tolerance of each strain to simulated gastrointestinal conditions.

Materials and Methods

Biochemicals
All biochemicals used were obtained from Sigma-Aldrich (Dorset, U.K).

Determination of gastrointestinal transit tolerance
An aliquot (10ml) of each probiotic culture (Yakult® Original, Yakult® Light, Actimel® Original and Actimel® Fat Free) was diluted with distilled water (1:10). To simulate gastric digestion at pH 2, 100µl of Hydrochloric Acid (32%) and Porcine Gastric Mucosa Pepsin (final concentration of 3mg ml^{-1}) were added at 0 hours. Samples were placed in a shaking water bath for 3 hours at 200rpm to respectively simulate residence time in the stomach and peristalsis. After 3 hours of simulated gastric digestion, 400µl of Sodium Hydroxide (4M), to increase the pH to 6.5, and Bovine Bile Salts (0.3% w/v) were added to simulate duodenal digestion. Samples were incubated in the shaking water bath for a further 2 hours to simulate residence time in the duodenum. Aliquots (1ml) were taken from each culture at 0, 3 and 5 hours immediately after any additions were made. Samples were serially diluted ten-fold using PBS, spread plated onto MRS Agar and incubated at 37^{o}C for 72 hours in a Carbon dioxide incubator (5%). Controls were performed in the same way, but without the additions of Hydrochloric Acid, Pepsin, Sodium Hydroxide or Bile Salts.

Statistical Analyses
For each probiotic product, a Two-sample *T*-test was used to determine if reductions in bacterial counts, after 5 hours of exposure to simulated gastrointestinal transit, differed significantly from controls. A General Linear Model was used to determine: 1) if reductions in bacterial count, during simulated gastric, duodenal and total gastrointestinal transit, differed significantly between probiotic brands (Yakult®/Actimel®) and 2) if reductions in bacterial count, during simulated gastric, duodenal and total gastrointestinal transit, differed significantly between versions of probiotic brands (Original/Light). A second General Linear Model was used to determine if bacterial count reductions of each strain after simulated gastric transit and after simulated duodenal transit differed significantly.

Results

Overall effect of simulated gastrointestinal transit on bacterial viability
Each strain, within all 4 probiotic products, retained viability after 5 hours of exposure to simulated gastrointestinal conditions, as shown in Figure 1. Mean final counts ranged from 6.50 $\log_{10}$ CFU ml^{-1}, by *L. casei* Shirota in Yakult® Light, to 8.36 $\log_{10}$ CFU ml^{-1} by *L. casei* DN-114 001, in Actimel® Fat Free.

Results of a Two-sample *T*-test indicated that the overall reduction in bacterial count of *L. casei* Shirota, within Yakult® Original (1.000 ± 0.157; Mean ± Standard Deviation), after 5 hours of simulated gastrointestinal transit, was significantly greater ($T = 9.15$; $P<0.05$) than the reduction in bacterial count observed in its control (0.078 ± 0.077). Similarly, the overall reduction in bacterial count of *L. casei* Shirota within Yakult® Light (0.986 ± 0.317), after 5 hours, was significantly greater ($T = 5.35$; $P<0.05$) than the reduction in bacterial count in its control (-0.006 ± 0.056). The overall reduction in *L. casei* DN-114 001, within Actimel® Fat Free (0.428 ± 0.059), was also significantly greater ($T = -6.35$; $P<0.05$) compared with its control (-0.035 ±

0.112) but was notably lower than the overall reduction of *L. casei* Shirota in both Yakult® Original and Yakult® Light.

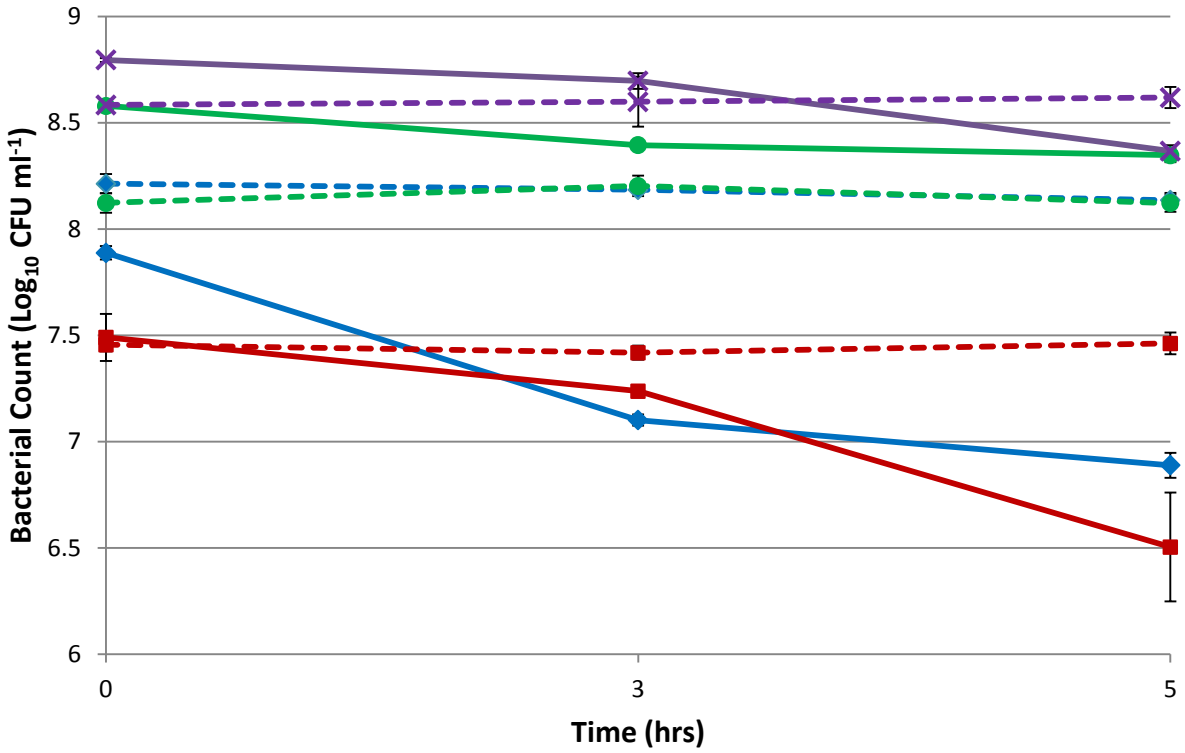

Figure 1: Survival of *L. casei* Shirota and *L. casei* DN-114 001, within Yakult® and Actimel® products, during simulated gastrointestinal and control conditions.
Values are means from three replicates ± Standard Error of the Mean. Solid lines represent simulated gastrointestinal transit; 0-3 hours represents simulated gastric transit; 3-5 hours represents simulated duodenal transit. Dotted lines represent controls. ●: *L. casei* DN-114 001 within Actimel® Original; **X**: *L. casei* DN-114 001 within Actimel® Fat Free; ♦: *L. casei* Shirota within Yakult® Original; ■: *L. casei* Shirota within Yakult® Light.

L. casei DN-114 001 in Actimel® Original was the only strain not to exhibit a reduction in overall bacterial count, after 5 hours, which was significantly greater than its control.

Results of the first General Linear Model (shown in table 1) indicated that the reduction in bacterial count, after 5 hours of simulated gastrointestinal transit, was significantly different between brands ($F = 38.41$; $P<0.05$). Using Tukey comparisons, *L. casei* Shirota within both versions of Yakult® exhibited a significantly greater mean reduction in number (0.993 ± 0.220) compared with *L. casei* DN-114 001 in versions of Actimel® (0.330 ± 0.129). Whether the product was Original or of a Light/Fat Free variety, had no significant effect on the overall reduction in bacterial count. After 5 hours of simulated gastrointestinal transit, *L. casei* Shirota within Yakult® Original demonstrated the greatest reduction in number (1.000 ± 0.157), with *L. casei* DN-114 001, within Actimel® Original, exhibiting the smallest bacterial count reduction (0.232 ± 0.095).

Effect of simulated gastric transit on bacterial viability

Results of the first General Linear Model (table 1) indicated that during simulated gastric transit (0-3 hours) the reduction in bacterial count was significantly affected

by both brand (F = 33.91; P<0.05) and version of brand (F = 22.73; P<0.05). *L. casei* Shirota within Yakult® products exhibited, on average, significantly greater reductions (0.520 ± 0.319) in bacterial count than *L. casei* DN-114 001 within Actimel® products (0.141 ± 0.081) after 3 hours of simulated gastric conditions. Lactobacilli strains within Original versions of each probiotic exhibited significantly greater reductions in number (0.485 ± 0.340) than strains contained within Light/Fat Free versions (0.176 ± 0.144). *L. casei* Shirota within Yakult® Original demonstrated the greatest reduction in number (0.787 ± 0.102) with *L. casei* DN-114 001, within Actimel® Fat Free, demonstrating the lowest reduction (0.099 ± 0.070), when compared with all probiotic versions.

Table 1: Differences in mean reduction of bacterial count between probiotic brands and versions, during gastric, duodenal and total gastrointestinal transit

	Least-Squares Mean Reduction of Bacterial Count* (log_{10} CFU ml^{-1})		
	Gastric transit (0-3 hours)	**Duodenal transit (3-5 hours)**	**Total gastrointestinal transit (0-5 hours)**
Yakult® Original	0.78686[a]	0.21290	0.99976[a]
Yakult® Light	0.25241[b]	0.73347[a]	0.98589[a]
Actimel® Original	0.18383[b]	0.04804[b]	0.23187[b]
Actimel® Fat Free	0.09859[b]	0.32966	0.42825[b]
Main Effect of BRAND			
Yakult®	0.51964[a]	0.47319[a]	0.99282[a]
Actimel®	0.14121[b]	0.18885[b]	0.33006[b]
Main Effect of VERSION			
Original	0.48535[a]	0.13047[a]	0.61581
Light/Fat Free	0.17550[b]	0.53156[b]	0.70707

Within each outlined group, values that do not share a lowercase letter are significantly different; P<0.05.*Of L. casei Shirota within Yakult versions and of L. casei DN-114 001 in Actimel versions.

Effect of simulated duodenal transit on bacterial viability

Parallel to gastric transit tolerance, during simulated duodenal transit (3-5 hours) reduction in bacterial count was significantly different between brands (F = 5.93; P<0.05) and versions of brands (F = 11.80; P<0.05) as shown in table 1. Once again, reductions in bacterial count of *L. casei* Shirota within both versions of Yakult® (0.473 ± 0.316) were significantly greater, on average, than reductions of *L. casei* DN-114 001 within both Actimel® versions (0.189 ± 0.156). In contrast to gastric transit tolerance, Lactobacilli strains within Light/Fat Free versions demonstrated significantly greater reductions (0.532 ± 0.258) than strains within Original versions (0.130 ± 0.098). *L. casei* Shirota within Yakult® Light demonstrated the greatest reduction in number (0.733 ± 0.207) with *L. casei* DN-114 001 in Actimel® Original exhibiting the smallest reduction (0.048 ± 0.021).

The mean reductions in bacterial count after simulated gastric and duodenal transit are shown in Figure 2, illustrating the elevated susceptibility of strains within Original versions to simulated gastric transit and of strains within Light/Fat Free versions to

simulated duodenal conditions. A second General Linear Model, used to determine if reductions in bacterial count differed significantly between simulated gastric and duodenal transit, rendered the overall differences insignificant.

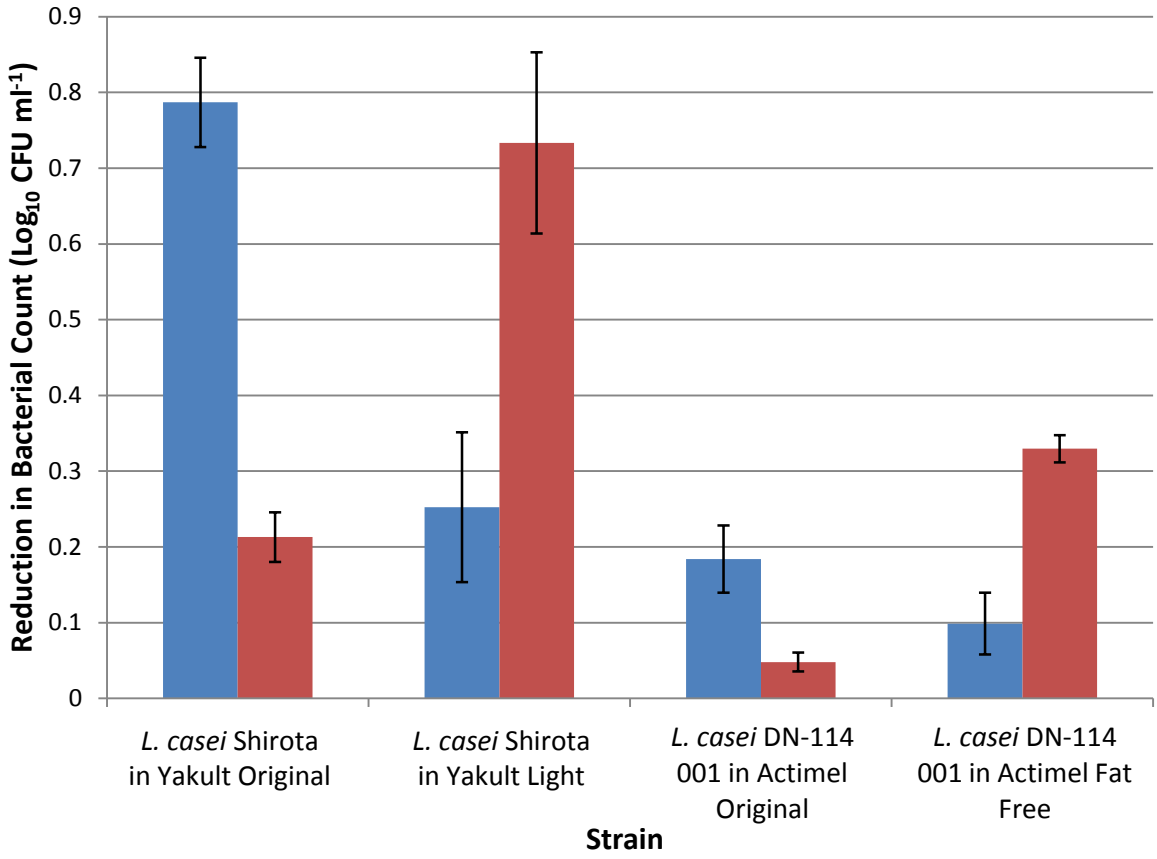

Figure 2: Reductions in Bacterial Counts after Simulated Gastric and Duodenal Transit. Values are means from three replicates ± Standard Error of the Mean. Blue bars (left) represent simulated gastric transit; red bars (right) represent simulated duodenal transit.

Discussion

Ingestion of Yakult® and Actimel® products has become increasingly popular within current society, as a result of their vast range of proposed probiotic benefits and respective claims to "top up and support the work of beneficial bacteria in the gut" and "help strengthen natural defences" (Danone Actimel, 2009a; Yakult, 2006a). However, in order for these products to exert their proposed health-promoting effects, the *Lactobacillus casei* strains within must overcome the inhospitable conditions of the human gastrointestinal tract, and remain abundant, viable and active enough to subsequently colonise the intestinal epithelia. This study assessed the capability of *L. casei* Shirota, within commercialised products Yakult® Original and Yakult® Light, and *L. casei* DN-114 001, within Actimel® Original and Actimel® Fat Free, to survive simulated *in vitro* gastric and duodenal transit. The effect different food matrices had on the survival rates of bacteria was determined and furthermore, if strain tolerance to simulated gastric conditions differed significantly from strain tolerance to duodenal conditions. This is the first known study to evaluate the tolerance of *L. casei* Shirota and *L. casei* DN-114 001 to *in vitro* gastric and subsequent duodenal transit within their commercialised food matrices.

Significantly greater ($P<0.05$) *in vitro* tolerance to simulated gastrointestinal transit (gastric plus duodenal digestion) was exhibited by *L. casei* DN-114 001 when compared with *L. casei* Shirota. This is consistent with previous *in vivo* trials which demonstrated that *L. casei* DN-114 001 has the capacity to survive throughout the human intestinal tract (Oozeer *et al*, 2006; Tormo-Carnicer *et al*, 2006). Although results of this study demonstrate that tolerance of *L. casei* Shirota to simulated gastrointestinal transit is poor and significantly lower ($P<0.05$) than that of *L. casei* DN-114 001, it also demonstrates the capability of this strain to survive and retain viability in these conditions. Therefore, even though simulated gastrointestinal conditions results in a significant reduction of *L. casei* Shirota, it could potentially still reach its site of action alive to confer its proposed probiotic benefits. This capability of *L. casei* Shirota to survive *in vitro* gastrointestinal transit is supported by earlier *in vivo* human feeding trials (Spanhaak *et al*, 1998; Tuohy *et al*, 2007).

In order to reach the intestines, potentially probiotic bacteria must first survive passage through the stomach. Over 2 litres of gastric juice, with a pH as low as 1.5, is secreted by parietal cells into the stomach each day (Morelli, 2000). Hydrochloric acid, the main constituent of gastric juice, helps destroy ingested microorganisms and stimulates the activation of pepsin, which degrades proteins into peptides. In conjunction, these secretions provide a powerful barrier to the survival of ingested probiotic bacteria. Species of the genus *Lactobacillus* have long been regarded as acid tolerant; however, gastric resistance has been shown to be highly strain-dependent, with great variations between strains of the same species (Tannock, 2004). This is supported by the results of this study, with *L. casei* DN-114 001 showing significantly ($P<0.05$) higher resistance to simulated gastric transit, than *L. casei* Shirota. This higher resistance of *L. casei* DN-114 001 to gastric conditions may be attributable to elevated enzyme F_0F_1-ATPase activity which has been shown to be present in certain *Lactobacillus* strains (Corcoran *et al*, 2005). This enzyme increases a bacterium's tolerance to highly acidic conditions by generating a proton motive force across the cell wall, increasing the cell's intracellular pH when extracellular pH is low (Fortier *et al*, 2003). Poor tolerance of *L. casei* Shirota to gastric conditions is consistent with results obtained by Guo *et al* (2009) who demonstrated that the survival rate of *L. casei* Shirota significantly dropped after 4 hours of exposure to simulated gastric juice at pH 2.5. Contrarily, Curto *et al* (2011) reported that the concentration of secreted lactic acid (a good indicator of bacterial survival) and percentage recovery of *L. casei* DN-114 001, during simulated gastric digestion, was generally lower, compared with *L. casei* Shirota, indicating poorer adaptation of the bacterium to its environment. A possible reason for these contradictory results is the differences in duration of simulated gastric digestion in each study, with Curto *et al* (2011) exposing strains to simulated gastric conditions for 1 hour, compared with a more realistic 3 hours in this study. Thus, the true capability of each strain to adapt to gastric conditions was not adequately assessed. Findings of this study are also inconsistent with results obtained by Maragkoudakis *et al* (2006) whom reported complete loss of viability of both strains after 3 hours of exposure to pH 2 and pepsin. However, this can be clearly attributed to the strains not being assessed within their commercialised food matrices, which have been shown to confer protection, to some extent, via the presence of milk substances. Milk and milk proteins, which are present in all Yakult® and Actimel® products, have been shown to improve gastrointestinal tolerance of Lactobacilli species; primarily decreasing their susceptibility to gastric transit and thus increasing their survival rate (Charteris *et al*, 1998; Curto *et al*, 2011).

After passage through the stomach, probiotic bacteria must then survive duodenal transit, which is arguably less influential on bacterial viability than gastric transit (Holzapfel *et al*, 1998). However, this has not been supported by findings of this study as no overall significant difference between bacterial reduction after gastric transit and bacterial reduction after duodenal transit was observed. The capability of a bacterial probiotic to resist the action of bile is considered an imperative trait. Bile is secreted from the gall bladder into the duodenum where it acts in conjunction with digestive enzymes to emulsify and digest lipids. Comparable to the degree of gastric tolerance demonstrated by each strain, *L. casei* Shirota exhibited a significantly greater ($P<0.05$) reduction in number, during simulated duodenal digestion, and therefore significantly lower tolerance to these conditions than *L. casei* DN-114 001. Differences in Bile Salt Hydrolase (BSH) activity (an enzyme which deconjugates and decreases the digestive capability of bile) may explain the variation in resistance to duodenal conditions between strains. However, Maragkoudakis *et al* (2006) reported partial bile salt hydrolysis by *L. casei* Shirota and no bile salt hydrolysis by *L. casei* DN-114 001; contradictory of results obtained in this study. Results acquired by Curto *et al* (2011) are one of few which can be effectively compared to results of this study, as tolerance to duodenal digestion is assessed immediately following simulated gastric digestion, with no removal or replacement of strains between stages. Supportive of findings in this study, *L. casei* DN-114 001 in milk, showed higher percentage recovery after gastric plus 1 and 2 hours of duodenal digestion than *L. casei* Shirota; although the differences were rendered insignificant.

Observed differences in strain tolerance, to simulated gastric and duodenal transit, may not be simply attributable to their capability to adapt, but may be correlated with differences in food matrix composition. As discussed previously, food matrix composition, particularly the presence of milk substances, increases the capacity of strains to survive the gastrointestinal tract. However, further correlations between the nutritional content of each commercialised food matrix and bacterial survival have also been observed. Results of the Two-Sample *T*-tests revealed that the reduction in number of *L. casei* DN-114 001, within Actimel® Original, after 5 hours of simulated gastrointestinal transit, was not significant compared to its control, whereas the reduction of the same strain, within Actimel® Fat Free, after 5 hours was significant ($P<0.05$). The composition of Actimel® Original, which contains higher levels of fat and carbohydrate, must therefore confer a greater degree of protection to *L. casei* DN-114 001 than Actimel® Fat Free (Danone Actimel, 2009b).

The significant differences ($P<0.05$) observed between gastrointestinal tolerance of *L. casei* Shirota, within Yakult® products, compared to *L. casei* DN-114 001, within Actimel® products, may also be potentially attributable to the differences in food matrix composition. For example, Actimel® versions contain over twice as much protein as Yakult® versions; providing a greater proportion of non-bacterial proteins for hydrochloric acid and pepsin to act on during gastric transit (Danone Actimel, 2009b; Yakult, 2006b). Furthermore, Actimel® products additionally contain yoghurt cultures, which also confer some protection against simulated gastric conditions. In conjunction, these components of Actimel® food matrices decrease the susceptibility of *L. casei* DN-114 001 to simulated gastric transit and hence, increase its chance at surviving subsequent simulated duodenal transit.

When comparing food matrix composition of Original and Light/Fat Free versions of each brand, no significant correlations with overall bacterial count reductions were

observed. However, when simulated gastric and duodenal conditions were regarded separately, whether the matrix was of an Original or Light/Fat Free variety significantly affected bacterial survival. Strains within Original versions of Yakult® and Actimel® exhibited significantly ($P< 0.05$) greater reduction in numbers and thus significantly lower tolerance to simulated gastric conditions than strains within Light/Fat Free versions. Therefore, the composition of Light/Fat Free versions, of Yakult® and Actimel®, confer a greater protective effect to strains contained within each product, than the composition of Original versions, when exposed to simulated gastric conditions. In contrast to gastric tolerance, strains within Light/Fat Free versions of each brand experienced significantly greater ($P<0.05$) reductions in number and thus, significantly lower tolerance to duodenal conditions, than strains contained within Original versions. This is likely to be attributable to the higher fat content of Original versions compared to Light/Fat Free versions; providing more non-bacterial lipids for bile to act on (Danone Actimel, 2009b; Yakult, 2006b).

The major strength of this study is that it takes into account the food matrices each bacterium is sold, and claims to confer benefits, within. In previous studies, the bacterium is typically isolated before its survivability during simulated gastrointestinal transit is assessed. A further important strength of this study is the follow-on of simulated duodenal transit immediately after simulated gastric transit; imitating *in vivo* human gastrointestinal digestion to a greater degree. By assessing a strain's tolerance to gastric and duodenal conditions separately, the majority of previous studies have led to misleading predictions of a strain's probiotic potential. Furthermore, the duration of exposure to simulated gastric and duodenal conditions, of respectively 3 and 2 hours, are more realistic of *in vivo* human digestion compared with the duration's used in earlier studies. Hence, a more accurate prediction of a strain's ability to survive *in vivo* gastrointestinal transit is achieved. The inclusion of pepsin in this study also improves the degree of similarity between *in vitro* and *in vivo* human gastric conditions.

However, a number of limitations of the methodology used in this study contribute to notable dissimilarities to *in vivo* human gastrointestinal conditions. For instance, the bile and pepsin used are of bovine and porcine origin respectively, which may have greater or lower digestive capability compared to those of human origin. Furthermore, the pH of the stomach varies greatly throughout digestion and can reach as low as 1.5. Thus, to effectively determine the survivability of each strain during gastric transit, the strains should have been exposed to a broader pH range. The secretion of pancreatin, which consists of digestive enzymes trypsin, amylase and lipase, into the duodenum is also overlooked during this study; notably increasing the chance of each strain's survival during simulated duodenal digestion. Even though the survivability of each strain was assessed, their aptitude to adhere to intestinal cells was not. Thus, although all strains retained viability after 5 hours of simulated gastrointestinal transit, with *L. casei* DN-114 001 (within Actimel Original) demonstrating resistance to gastrointestinal conditions, they may be incapable of colonising the intestinal tract and hence, incapable of bestowing their suggested therapeutic benefits.

In conclusion, strains contained within commercialised Yakult® and Actimel® products of Original and Light/Fat Free varieties retained viability after 5 hours of exposure to simulated gastrointestinal transit. Whether the product was of an Original or a Light/Fat Free variety did not have an overall significant effect on the

survival of each strain, but did when gastric and duodenal tolerance was examined separately; possibly due to the variation in nutritional content and focus of gastrointestinal secretions. The survival of *L. casei* Shirota within both Yakult® Original and Yakult® Light was significantly affected during simulated gastrointestinal transit, compared with the survival of *L. casei* DN-114 001 within Actimel® Original and Actimel® Fat Free, suggesting *L. casei* Shirota has poorer tolerance to gastrointestinal conditions. However, when compared to control results, the survival of *L. casei* DN-114 001 within Actimel® Fat Free was significantly affected by gastrointestinal conditions, whereas survival of the same strain within Actimel® Original was not significantly affected; a strong implication that food matrix composition is significantly correlated with bacterial survival. Out of the four probiotic products assessed; *L. casei* DN-114 001, contained within Actimel® Original, demonstrated resistance to simulated gastrointestinal conditions. However, this does not denote probiotic functionality as notable dissimilarities between *in vivo* gastrointestinal tract conditions and the *in vitro* conditions employed in this study remain. Furthermore, despite the fact that all strains retained viability, their capacity to adhere to intestinal epithelia and bestow their proposed therapeutic benefits has not been confirmed.

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